isolating transformants identified as having no detectable extracellular high alkaline protease.

28. (New) A method for producing an alkalophilic asporogenic *Bacillus* novo species PB92 of minimal indigenous extracellular protease level, said method comprising:

transforming an alkalophilic asporogenic *Bacillus* strain with a specifically-mutated *Bacillus* novo PB92 alkaline protease.

29. (New) An alkalophilic *Bacillus* strain which is substantially incapable of reversion and which is substantially free of expression product of an indigenous extracellular alkaline protease gene.--

REMARKS

The Invention

The claimed invention is directed to methods and compositions for preparation of mutant high alkaline proteases and mutant alkalophilic *Bacillus* strains which produce only the mutant high alkaline protease and not the corresponding indigenous protease. Also claimed are a detergent composition comprising as an active ingredient one or more high alkaline proteases in a detergent composition or a laundry process.

The Pending Claims

Prior to entry of the above amendments, Claims 4-7, 9-17, 19, and 23-26 were pending in the present application. Claims 23, 4-7, 9-11, 26 and 28 (new) are directed to methods for production of a mutant high alkaline protease; Claims 12-13 and 27 (new) are directed to a method of

obtaining an alkalophilic *Bacillus* strain having a reduced extracellular alkaline protease level; Claims 14-16 and 29 (new) are directed to an alkalophilic *Bacillus* strain producing a mutant high alkaline protease; Claim 17 is directed to mutant high alkaline protease; Claim 19 is directed to a detergent composition comprising as an active ingredient high alkaline protease. Claim 24 is directed to a method of preparing a detergent composition comprising high alkaline protease as an active ingredient. Claim 25 is directed to a method of processing laundry with the claimed detergent composition.

<u>The Office Action</u>

Claim 17 is provisionally rejected both under the obviousness-type double-patenting doctrine as being unpatentable over Claims 60-66, 70-73, and 75-82 of co-pending application Serial No. 07/427,103 and under 35 U.S.C. § 103 as being obvious over that same application.

Claims 4-7, 9-17, 19, and 23-26 are rejected under 35 U.S.C. § 112, first paragraph, asserting that the disclosure is enabling only for claims limited to methods of producing an alkalophilic asporogenic *Bacillus* novo species PB92 of minimal indigenous extracellular protease level, transformed with a specifically-mutated *B.* novo PB92 alkaline protease.

Claims 12, 15-16, 19, and 24-26 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention.

The amendment filed 9/7/93 is objected to under 35 U.S.C. § 132 because it allegedly introduces new matter into the specification.

Claims 4-7, 9-17, 19, and 23-26 are rejected under 35 U.S.C. § 103 as being unpatentable over **Fahnestock et al.** and **Estell et al.**, in view of **TeNijenhuis** and **Suggs et al.**

The Amendments

The specification is amended to correct certain clerical errors. No new matter is introduced by these amendments.

Claim 12 is amended to recite positively that the alkalophilic *Bacillus* strain encodes a replication function, to provide antecedent basis for the phrase in that claim "whereby the replication function encoded by said vector".

Claim 14 is amended to include dependency from the method according to new Claim 27 and to delete the language objected to by the Examiner as constituting new matter.

Claims 15 and 26 are amended to correct clerical errors occurring in the originally filed claims.

Claim 17 is cancelled.

Claims 19 and 24-25 are amended to clarify that the "one or more" language refers to "one or more mutant forms of a high alkaline protease exhibiting altered protease activity."

Claim 23 is amended to included an alkalophilic *Bacillus* strain host substantially incapable of reversion. Support for this amendment is found in the specification; specifically on page 7, lines 16-17.

Claim 27 is added to include a claim directed to illegitimate recombination, which language was in originally filed Claim 16. Support for this new claim is found generally in the specification; specifically on page 9, lines 15-16. No new matter is presented by this new claim.

Claim 28 is added to recite Applicants' invention indicated as enabled by the Examiner on page 3, lines 17-20 of the outstanding Office Action. No new matter is presented by this new claim.

Claim 29 is added to include a claim directed to an alkalophilic *Bacillus* strain which is non-reverting and which does not produce indigenous extracellular alkaline protease. Support for the claim is found in the specification; specifically on page 6, lines 19-20 and on page 7, lines 16-17.

All pending claims, included non-amended claims, are listed in Appendix A for the Examiner's convenience.

Response to the Rejections

In the remarks that follow, the Examiner's specific objections and rejections are indented and presented in bolded small print, followed by Applicants' response.

> Claim 17 is provisionally rejected under the obviousness-type double patenting doctrine as being unpatentable over Claims 60-66, 70-73 and 75-82 of copending application Serial No. 07/427,103. Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant broadly-claimed "mutant high alkaline protease" encompasses the specifically claimed changes to the protease of the '103 application.
>
> This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Applicants herein cancel Claim 17, thus making this provisional rejection moot.

35 U.S.C. § 103

> Claim 17 is provisionally rejected under 35 U.S.C. § 103 as being obvious over copending application Serial No. 07/427,103.
>
> Copending application Serial No. '103 has a common inventor with th instant application. Based upon the earlier effective

U.S. filing faster of the copending application, it would constitute prior art under 35 U.S.C. 102(e) if patented. This provisional rejection under 35 U.S.C. § 103 is based upon a presumption of future patenting of the conflicting application.

Applicants herein cancel Claim 17, thus making this provisional rejection moot.

35 U.S.C. § 112, first paragraph

Claims 4-7, 9-17, 19, and 23-26 are rejected under 35 U.S.C. § 112, first paragraph, as the disclosure is enabling only for claims limited to methods of producing an alkalophilic asporogenic Bacillus novo species PB92 of minimal indigenous extracellular protease level, transformed with a specifically-mutated B. novo PB92 alkaline protease. See M.P.E.P. §§ 706.03(n) and 706.03(z).

The claims are not properly enabled for the recitation of a "mutant high alkaline protease", and specifically, claim 17 is further not enabled for such proteases "differing in at least one amino acid from a wild-type high alkaline protease." Applicants' arguments filed in all responses have been considered but are not deemed persuasive. Applicants have stated that "it would be well within the skill of one of ordinary skill in the art to determine which mutations would result in a protease differing by at least one amino acid from the indigenous protease". This is not deemed persuasive, as again, one skilled in the art would not be able to determine what type of mutation, how many, at what amino acid (nucleotide on the gene), etc., including all of the millions of variations possible in order to fulfill what the applicants regard as the invention. More importantly, one skilled in the art could not prophetically predict the outcome of any mutation upon the gene, the enzyme produced, and their resultant effect upon the instantly claimed invention. This would require undue experimentation, primarily due to the unpredictable nature of the art, and the scarcity of guidance and/or working examples in the specification.

To continue, applicants have stated, at page 5-7 of the response filed 9-7-93, that "a knowledge of protease gene structure before or after mitogenesis and the effect of gene mutation on protease activity is not essential to practice the instant invention" (pg. 7). This is not deemed persuasive, as, due to the unpredictable nature of the art, supported by applicants urging of blindly producing any mutation in the protease gene, any unknown and random mutation could and would be expected to have a delirious effect upon the protease itself, or the Bacillus organism hosing such. Improper tertiary folding, steric hinderance, or negative effect upon the active site are just a few of the probable possibilities that would be expected to

occur to the gene itself. In addition, there is no safe predictability of what effect the mutated protease would have upon the host cell itself, or even if the gene would be translated at all.

The claims are not properly enabled for the recitation of any "mutant high alkaline protease", and any "alkalophilic Bacillus strain". Applicants have stated that the strain PB92 has been used merely as an example, and that the specification provides enablement for the use of other types of these strains, and for other "mutant high alkaline proteases". Applicants further state that techniques for such are "routine and require no inventive skill or undue experimentation" (pg. 7, response of 9-7-93). This is not deemed persuasive for the reasons of record. Primarily, the specification has not provided pertinent information regarding any other "high alkaline protease" gene, nor any appropriate Bacillus strain that would satisfy the requirements of the invention. This fact is important, as the claims are not commensurate in scope with the specification and its enablement. This information is essential to the function of the claimed invention, and the essential material may not be improperly incorporated into the specification, and does not find support within the teachings of the specification. Thus, one skilled in the art would in no way be enabled to practice the claimed invention with any such gene or strain other than the enabled Bacillus PB92.

The specification is not properly enabled for claims to any "derivative thereof" of a Bacillus novo species PB92. This phrase encompasses predetermined and random mutants of the strain, and progeny of the strain that may or may not contain the gene for the "mutant high alkaline protease" and/or a revertant strain with the indigenous gene. The specification does not properly teach nor describe to one skilled in the art these "derivatives", nor how to obtain and/or use such. Thus, this results in undue experimentation for one skilled in the art to attempt to produce such without proper guidance from the specification.

The method of claim 12 is not properly enabled by the specification. The claimed invention is not reflective of the method and "conditions whereby the replication function encoded by said vector is inactivated". It would require an inordinate amount of experimentation for one of ordinary skill to attempt to determine what and where the "replication function" of the vector is, its relationship to the rest of the invention, and method of "inactivating" such, and its possible effects upon the instant invention. Further, the specification is not enabling for any or all possible methods of "identifying" transformants with no detectable indigenous protease. The claims are not commensurate in scope with the enablement of the specification for such methods.

Applicants respectfully traverse this rejection. Applicants note that Claim 17 is herein cancelled, thus any rejection of that claim now is moot.

The cited MPEP sections do not support the present rejection. Section 706.03(n) simply requires *correspondence* between the language of the specification and the language of the claims, and it is not disputed that this requirement is met in the present application. Section 706.03(z) requires that the recitation of a *genus* of compounds in a claim (e.g., *Bacillus*) be supported by the *disclosure* of a sufficient number of *species* in the specification (e.g., PB92, PBT110, PBT110-INTS, PBT125, PBT126). It is not a requirement for any number of working examples. It is respectfully submitted that the extensive disclosure of the PB92 and the PBT110-derived mutants in the specification at pages 12-18 is more than adequate to support the genus of *Bacillus* recited in the pending claims.

Furthermore, it is well-established that to be enabling, the specification need only provide sufficient information to allow one skilled in the art to make and use the invention without undue experimentation. Scripps Clinic & Research Foundation v. Genentech, Inc., 927 F.2d 1565, 18 USPQ2d 1001, 1006 (Fed. Cir. 1991). "That *some* experimentation may be required is not fatal; the issue is whether the amount of experimentation required is 'undue.'" In re Vaeck, 947 F.2d 488, 495, 20 USPQ2d 1438, 1444 (Fed. Cir. 1991) [emphasis in original]. Further, the specification need not teach what is well known in the art. In re Wands, 858 F.2d 731, 735, 8 USPQ2d 1400, 1402 (Fed. Cir. 1988).

In this case, the Examiner is ignoring the teachings in the art and in the specification that would lead one skilled

in the art who is in possession of the specification to be able to use the claimed methods. Claim 23, for example, is directed to a method for production of a mutated high alkaline protease "substantially free of indigenous extracellular high alkaline protease." The method identified the preferred host as an "alkalophilic *Bacillus* strain host having no detectable indigenous extracellular protease and substantially incapable of reversion."

One aspect of the invention is that it overcomes significant problems previously encountered with the use of *Bacilli* as production hosts for recombinant proteins. *Bacilli* produce and secrete a variety of proteases which tend to degrade the heterologous proteins produced. Approximately 90% of this proteolytic activity can be attributed to neutral metal protease (NPR) and serine protease (APR). While several approaches have been used to overcome this latter problem, the resulting organisms were not completely devoid of extracellular proteolytic activity. Additionally, it was thought that the use of asporogenous mutants for the production of recombinant proteases would be unsatisfactory as the introduction of a sporulation deficiency in a strain would be expected to result in decreased protease production. (See, specification p. 1, ln. 31 - p. 2, ln. 19.)

Applicants have solved these problems with the claimed invention, so that not only may a mutated high alkaline protease be produced which is substantially free of indigenous extracellular high alkaline protease, but the mutated enzyme is surprisingly produced in large quantities, even when the host is an asporogenous *Bacillus*. Thus, it is not production of a mutant enzyme *per se* that is significant, but that it can be produced in the absence of

indigenous protease. How to accomplish this is set forth in the specification in general terms on page 7, line 12, through page 10, line 10. The technique is exemplified *in detail* in the Experimental section beginning on page 15, line 26.

The methodology, once worked out as described by Applicants, is applicable to *Bacilli* other than those used in the examples. Indeed, the specification is quite clear that this process is not limited to use with a PB92 *Bacillus* host strain, which the specification describes as "an example" of the invention. (Specification, p. 12, ln. 8). Asporogenous mutant *Bacillus* strains are a further example of a group of *Bacilli* that may be used, of which the group "derived from PBT110 and its derivatives" is preferred. (Id., p. 12, ln. 29-33). Other examples are suggested in the specification. (Id., p. 12, ln. 10-21.)

The Examiner is wrong, as a factual matter, in stating that the specification is limited to one particular example. The specification in fact gives several examples, and clearly identifies such examples as merely preferred embodiments of an invention that is effective for an entire class of *Bacillus* strain hosts. The Examiner has provided no substantiation for his assertion that the claimed methods could not be used with *Bacilli* other than those exemplified. Once the technical problem of making the host cell which does not produce extracellular high alkaline protease was solved by the Applicants, the application of the solution to other host organisms is well within the ordinary skill of the art. Transforming the host *Bacillus* with the constructs encoding for the gene for the protease to be produced and growing the cells to produce the protease, are within the skill in the art (see the references of record, such as

Fahnestock et al.) as are methods for producing mutated genes (see, for example, **Gorman et al.**, also of record). What was not within the skill of the art prior to Applicants' subject invention, was preparation of the host cell in which the entire indigenous protease gene had been deleted so as to produce an organism which did not produce extracellular high alkaline protease.

The Examiner also is wrong as a legal matter in attempting to limit Applicants' claims to the preferred embodiment disclosed in the specification. It was settled long ago that:

> To demand that [Applicant] shall limit his claims . . . to materials which meet the guidelines specified for "preferred" materials in a process . . . would not serve the constitutional purpose of promoting progress in the useful arts.

In re Goffe, 542 F.2d 564, 567, 191 USPQ 429, 431 (C.C.P.A. 1976). The Federal Circuit has reaffirmed that principle in *In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988). In that case, the Court held that the PTO erred in rejecting an applicant's claim to methods of using a generic class of antibodies. The applicant in *Wands* had deposited only a cell line secreting one specific antibody. Nonetheless, the Court held that the applicant was entitled to a patent covering the use of the generic class because one skilled in the art could produce other antibodies falling within the generic class for use in the method. Id. at 740, 8 USPQ2d at 1406-07. The same is true here. The Examiner has provided no support for his assertion that the claimed invention would not be operative with alkalophilic *Bacillus* strains other than PB92, nor why it would not be apparent to one skilled in the art how to make and use the claimed

invention, nor why undue experimentation would be required to practice the claimed invention.

Applicants have included examples of derivatives by reference to EP-A-02841126 (see page 12, line 13 of the specification), and examples of mutants by reference to EP-A-0328299 (see page 12, line 21 of the specification). Furthermore, Applicants specifically disclose information regarding the vector. On page 8, line 25 through 9, line 25, Applicants disclose that the vector used "is preferably a plasmid". At that part of the specification, Applicants also describe methods for selection of transformants that may be used in practicing Applicants' claimed invention. In view of Applicants' extensive characterization of mutant high alkaline proteases, *Bacilli*, and vectors, Applicants assert that one skilled in the relevant art would be able to practice Applicants' claimed invention.

Simply because all genes encoding extracellular high alkaline proteases are unknown or not cloned does not mean that Applicants are limited to what they have exemplified. If that were true, others could with impunity use Applicants' inventive concept simply by substituting another protease gene as the target gene for removal, substituting a *Bacillus* other than PB92 as the expression host. Applicants have shown that their invention is operable as claimed and therefore is entitled to their generic claims.

The specification discloses that the process of claim 23 is effective for *any* mutant proteases. (Id., p. 12, ln. 22 - p. 14, ln. 3). Indeed, the specification discloses that the claimed method is not limited to expression of a mutant high alkaline protease, but that any polypeptide of interest may be expressed in the absence of indigenous extracellular high alkaline protease. (Id., p. 12, ln. 34 -

p. 13, ln. 3). The specification describes the mutant proteases which can be expressed: those in which at least one amino acid is different from the wild type protease. (Id. at p. 13, line 3-16).

The Examiner's objection that "one skilled in the art would not be able to determine what type of mutation, how many, at what amino acid (nucleotide on the gene), etc., including all of the variations possible . . . [and] could not prophetically predict the outcome of any mutation upon the gene" (Paper 24, p. 4, lns. 3-9) misses the point of Applicants' invention. Knowing the type of mutation induced in a particular protease gene or the effect of a mutation on protease function is not required in order to practice the instant prevention. One aspect of Applicants' invention is that *any* mutant protease can be produced more readily in a *Bacillus* strain ***with an indigenous protease level that is reduced*** **by deletion of the indigenous protease gene.** One skilled in the relevant art would be able to identify a mutation "rendering the expression host incapable of producing indigenous proteases" and that, as a consequence, "necessitates the replacement and/or inactivation of the wild-type gene from the genome of a parent mutant *Bacillus* strain cell", as stated in the specification at page 7, lines 12-15.

Furthermore, methods for producing a mutant protease differing in at least one amino acid are well known in the art. Several such methods are disclosed in the specification as examples. (Specification, pp. 15-17, 19-27.) The specification teaches how to achieve mutation, as the Examiner appears to concede; that is all that is required for the invention. Enumerating the particular mutant strains which could be made is beyond the scope of

the invention. Thus, Applicants have disclosed all that is required to make and use the invention, and have complied with the requirements of section 112, first paragraph.

The Examiner erred in applying that rejection to Claim 9. In the Office Action, the Examiner concluded that the specification is enabling only for claims limited to "methods of producing an alkalophilic asporogenic *Bacillus novo* species PB92 of minimal indigenous extracellular protease level, transformed with a specifically-mutated *B.* novo PB92 alkaline protease." (Paper 24, at p. 3, lns. 16-20).

This ground does not justify rejection of Claim 9. That claim is directed to a method of producing a *Bacillus* strain limited to a *Bacillus* novo species PB92 as the expression host, since it depends from method Claim 23. The Examiner has offered no reason for inclusion of this claim in the rejection, and the Examiner's rejection by its own terms does not apply to Claim 9. Certainly it would be within the skill of the art to mutate other protease genes of interest and to insert them into the altered PB92 strain in order to obtain production of the protease of interest in the absence of indigenous extracellular high alkaline protease.

> Claims 9, 14, 19 and 23-26 are rejected under 35 U.S.C. § 112, first paragraph, for the reasons set forth in the objection to the specification.

Since the outstanding Office Action does not cite an objection to the specification, this additional basis for rejecting Claims 9, 14, 19, and 23-26 are unclear. Applicants respectfully submit that the rejection of these claims under 35 U.S.C. § 112, first paragraph, is addressed in the above discussion.

Accordingly, Applicants assert that the pending claims are legally enabled, satisfying the requirements of 35 U.S.C. § 112, first paragraph.

35 U.S.C. § 112, second paragraph

> Claims 12, 15-16, 19 and 24-26 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.
>
> Claim 12, third section (paragraph), recites "whereby the replication function..." (is inactivated). It is not previously described within the claim what the "replication function" is, or that it is even present in the instantly claimed invention.
>
> Claim 15, line 2, the term "said" is incorrectly spelled as "aid". Further, the claim recites said "mutant" Bacillus strain of independent claim 14, yet claim 14 does not claim a "mutant" strain. The term "mutant" does not properly and definitely describe the transformed strain of claim 14.
>
> Claim 16 recites deletion of the gene of claim 15 (ultimately dependent upon claim 12) "by homologous or illegitimate recombination". The independent claim 12 only recites the method by "homologous recombination", and thus claim 16 is indefinite in its improper limitation.
>
> Claims 19 and 24-25 are indefinite in the recitation of the phrase "one or more" of the protease produced. Since only one (type of) protease is being produced in the independent claims, the claims are indefinite for the recitation of "one or more" (it is assumed that applicants do not intend for a single molecule of the protease to be "one").
>
> The method of claim 26 is indefinite, as it is a method for producing a protease by culturing the host strain, yet there is no "recovery step" involved for obtaining the protease from the host. This is a necessary step to the claimed invention, as the mere production of the protease in the strain can not result in the claimed protease being accessible.

Applicants respectfully note that the claims are amended to address this rejection. Specifically, Claim 12 is amended to provide an antecedent basis for the phrase "whereby the replication function encoded by said vector is inactivated."

The clerical errors in Claim 15, including the typographical error and the erroneous reference to a "mutant alkalophilic *Bacillus* strain", as noted in the Office Action are corrected.

Regarding Claim 16, that claim now incorporates the subject matter of newly added Claim 27. Claim 27 recites a method of obtaining an alkalophilic *Bacillus* strain wherein the indigenous genes is deleted by illegitimate recombination. Thus, amended Claim 16 now includes proper limitations.

Regarding Claims 19 and 24-25, those claims are amended to more clearly describe Applicants' claimed invention. As amended, those claims now recite that a detergent is combined with "one or more mutant forms of a high alkaline protease." The Examiner is correct in stating that Applicants do not intend for a single molecule of the protease to be considered "one".

Regarding Claim 26, Applicants respectfully assert that the claimed inventive method does not require a specific recovery step, since the host itself may serve as a delivery vehicle for the protease. There is no requirement that the protease be "recovered" from the host. Thus, the claim is accurate as presented. Accordingly, Applicants assert that the claims, as amended, now describe Applicants' claimed invention.

35 U.S.C. § 132

> The amendment filed 9-7-93 is objected to under 35 U.S.C. § 132 because it introduces new matter into the specification. 35 U.S.C. § 132 states that no amendment shall introduce new matter into the disclosure of the invention. The added material which is not supported by the original disclosure is as follows:
>
> [a protease] "exhibiting altered protease activity".

> This phraseology is not supported at page 29 or anywhere else in the specification. Page 29, at best, describes "altered" protease levels of production, but does not describe the proteases having different activity. Note that the use of a "mutant protease" does not necessarily result in one "exhibiting altered protease activity."

Applicants respectfully traverse the rejection as being improperly applied in the present case. The Examiner states that the phrase "exhibiting altered protease activity" is not supported in the specification, and that the data in the specification demonstrates altered levels of protease *production* but does not demonstrate altered protease *activity*.

Applicants assert that the data presented in Table 1, on page 29 of the subject specification, demonstrates changes in protease production (e.g., PBT110 at 100%, and PBT125 at 0% relative protease production). As stated at lines 14-16, on page 29 of the specification, "The specific *activities* of the protease mutants...were used to determine the *production* of protease per mg. of protein." [emphasis added] Applicants clearly stated the correspondence between the relative protease production data and specific activity of the protease mutants. Thus, the change in relative protease production does support language reciting altered (e.g., changed) protease activity. Applicants assert that the phrase "exhibiting altered protease activity" clearly is supported by the specification, but in order to further prosecution have deleted the phrase objected to by the Examiner.

No!!

<u>35 U.S.C. § 103</u>

> Claims 4-7, 9-17, 19, and 23-26 are rejected under 35 U.S.C. § 103 as being unpatentable over Fahnestock et al. and Estell et al., in view of TeNijenhuis and Suggs et al. The references and

rejection are herein incorporated as cited in a previous Office Action.

Applicants' arguments filed in response to this rejection have been fully considered but they are not deemed to be persuasive. Applicants have [stated] that Fahnestock et al. and Estell et al. would not lead one to the instant invention in light of the secondary references.

Initially, applicants have stated that "asporogenous *Bacilli* are surprisingly useful in obtaining high level expression of a mutant protease gene" (paper of 9-7-93, pg. 8). This has been considered, however, it is not deemed persuasive, as this is not properly reflected in the claimed invention. Very few of the claims recite an "asporogenic" *Bacillus* strain let alone do any reflect the "unexpected" properties purported by applicants. Furthermore, the PB92 alkaline protease from the original strain of *Bacillus* PB92 was "produced in surprisingly high yields", according to TeNijenhuis et al. (Re. 30,602, of record), column 1, line 50. Thus, the use of the same indigenous natural promoter system and overall production system of that of the *Bacillus* PB92 strain would have naturally been expected to produce a "high yield" of protease.

Applicants assert that the prior art differs, as for example, since Fahnestock et al. inserts a CAT fragment to inactivate the protease sequence, there is the possibility of reversion. Applicants have stated that because of this and the fact that Estell et al. merely uses chemical mutagenesis to partially delete the native gene, that the method claims and those to the strain would not have been obvious. This is not found persuasive for the reasons of record. Fahnestock et al. does indeed use homologous recombination to delete the original functional gene, as do applicants. The fact that Fahnestock et al. replaces that with an inactive (mutant) analogous gene is not evidence of unobviousness. On the contrary, applicants have performed a similar step in the instantly claimed methods and strains. Applicants have deleted the original gene (same as Fahnestock et al.), and have replaced it with a (non-specified) analogous "mutant" protease gene, similar to that of the reference.

The fact that Fahnestock et al. still produces extracellular proteases is not pertinent to the instant invention. Applicants themselves have not produced strains that do not produce any extracellular proteases, and thus do not differentiate from the prior art. Estell et al. only compliments this by a similar deletion, with the replaced flanking regions containing only a small portion of the original coding region of the protease. It would have been obvious to further delete the rest of the coding region, for the mere assurance of complete success of no protease activity. Estell et al. have shown that this method produces no (neutral) protease activity, and applicants method does not differ patentably form this by deleting the rest of the coding region. The deletion of ntire genes from a genome is well-known and characterized, and is almost as old as genetic engineering itself.

> Applicants' contention that the deletion of an entire gene is an inventive concept is not deemed persuasive. Furthermore, at page 8 of the amendment filed 9-7-93, applicants themselves have stated that "the genetic technique of homologous recombination is well known in the art and is not a unique contribution of the Fahnestock et al. patent." Applicants have not demonstrated any results that would have been unexpected, unobvious, or superior to that taught by the prior art, absent convincing evidence to the contrary.
>
> Again, the limitation of "an alkalophilic *Bacillus* strain" does not render the claim patentably distinct from the similar methods of Fahnestock et al. and Estell et al., *per se*. The systems are the same, and both used with *Bacillus* organisms, of which many are already alkalophilic. Further, the mutation of the strain to produce an "asporogenic" variant is obvious and well known in the art to do, and is easily obtained via classic UV mutation techniques, also described in Fahnestock et al. The fact that Estell et al. state that asporogenic *Bacilli* are "unsatisfactory" for production of heterologous proteins is not persuasive or particularly pertinent, as Fahnestock et al. preferably teach both how to make and use asporogenic *Bacillus* to produce heterologous proteins. Thus, the method and *Bacillus* strain claims are not deemed patentable in view of the prior art.

Applicants respectfully traverse the rejection for the reasons previously stated and for the following reasons.

Applicants' claimed invention is a method of obtaining "an alkalophilic *Bacillus* strain having no detectable extracellular high alkaline protease" (Claim 12), an "alkalophilic *Bacillus* strain producing a mutant high alkaline protease exhibiting altered protease activity and substantially free of expression product of an indigenous extracellular alkaline protease gene" (Claim 14), a "mutant high alkaline protease" produced according to the claimed method (Claim 17), and a method for production of "a mutated high alkaline protease exhibiting altered protease activity and substantially free of indigenous extracellular high alkaline protease" (Claims 23 and 26).

The claimed method, as recited in Claim 12, includes the step of "transforming an alkalophilic *Bacillus* strain with a cloning vector comprising the 5' and the 3' flanking

regions *but not the coding region* of gene coding for the high alkaline protease" [emphasis added]. The claimed method, as recited in amended Claim 23, includes the step of growing an alkalophilic *Bacillus* strain host "substantially incapable of reversion and having no detectable indigenous extracellular protease *as a result of deletion of the gene* for indigenous extracellular protease" [emphasis added]. Thus, Applicants' claimed method includes the *deletion* of the entire gene for indigenous extracellular protease.

One advantage of Applicants' claimed invention is that it decreases the probability for any functional reactivation of the gene. This reactivation is a significant drawback to the prior art methods, including those cited in the outstanding Office Action, as noted by Applicants on page 2, lines 20-34 of the subject specification.

Applicants have disclosed an unexpected property of *Bacilli*: deletion of the indigenous protease coding region *enhances* production of mutant alkaline protease. This is shown in Table 1, on page 29, lines 20-44 of the specification. Such unexpected results are evidence that Applicants' invention would not have been obvious to those skilled in the art. *In re Corkill*, 771 F.2d 1496, 1500, 226 USPQ2d 1885, 1888 (Fed. Cir. 1985).

By contrast to Applicants' claimed invention, none of the cited references, considered either alone or in combination teaches deletion of the entire gene for indigenous extracellular protease. Specifically, **Fahnestock et al.** disclose use of *Bacillus* strains having reduced levels of indigenous protease for expression and secretion of heterologous polypeptides or proteins. However, unlike the instant invention, **Fahnestock et al.** inactivate the indigenous protease by adding an inactivation CAT gene

sequence to the DNA of the host strain. The resulting *Bacilli* still produce extracellular protease, both serine and neutral proteases as shown in Table 2 in column 9, lines 55 through 68 of that reference.

The Examiner states that "Applicants themselves have not produced strains that do not produce any extracellular proteases." This is erroneous in fact. In Table 1, on page 29 of Applicants' specification, is shown two base strains were produced that have a relative protease production of about zero: PBT125; and DS12367. These strains, as shown, were used as the base strain for transformation in accordance with Applicants' claimed invention and do not produce extracellular alkaline protease.

Furthermore, there is no teaching or suggestion in **Fahnestock et al.** of the *high alkaline* protease of Applicants' claimed invention. Nor do any of the secondary references provide the missing teaching or suggestion to use Applicants' claimed *high alkaline*, as compared with normal serine, protease.

The combination of **Fahnestock et al.** with the secondary reference of **Estell et al.** similarly do not teach Applicants' claimed invention. **Estell et al.** disclose preparation of a *B. subtilis* strain BG84 (column 20, beginning at line 27), using N-methyl-N'-nitro-N-nitrosoguanidine (NTA) mutagenesis of *B. subtilis* I168. BG84 is sporulation deficient. As stated in Applicants' previously filed response, the **Estell et al.** reference was not enabling for that which it is cited, since it does not teach one skilled in the art how to obtain alkalophilic *Bacilli* in which the gene for extracellular high alkaline protease was deleted. Furthermore, that reference discloses deletions *within* the protease gene, and not deletion of the

entire gene. Nothing in the **Estell et al.** reference suggests any reason to delete the rest of the coding region or any method for so doing.

Nothing in either **Fahnestock et al.** or **Estell et al.**, either alone or in combination, teaches or suggests the use of an alkalophilic *Bacillus* as an expression host. In fact, it is Applicants' disclosure of the unexpected property of *Bacilli*, namely, that deletion of the indigenous protease coding region actually enhances production of mutant alkaline protease, that the Examiner improperly is using to combine the **Fahnestock et al.** and the **Estell et al.** references. In fact, **Estell et al.** teach away from using an alkalophilic *Bacillus*, and in particular the use of asporogenic *Bacillus* strains as expression hosts.

As stated above, Applicants' claimed invention includes the step of growing an alkalophilic *Bacillus* strain host "substantially incapable of reversion". Support for the claim language may be found in the specification, for example at page 7, lines 16-17. There is nothing in any of the cited references to suggest this property of a *Bacillus* strain host. In fact, the expectation of one skilled in the relevant art is that reversions will occur. Thus, it would be expected that the mutants described in each of the cited references would undergo some degree of reversion. This is in contrast with Applicants' claimed invention, wherein the entire gene is deleted, leaving nothing to revert.

Regarding Applicants' Claim 5, directed to an asporogenic alkalophilic *Bacillus* strain, Applicants reassert that **Estell et al.** teach away both from the Applicants' claimed invention, and from the combination of that reference with the other references, including **Fahnestock et al.** Specifically, **Estell et al.** conclude that

"asporogenous [*Bacilli*]...are unsatisfactory for the recombinant production of heterologous proteins because asporogenous mutants tend to lyse during earlier stages of their growth cycle." Applicants respectfully disagree with the assertions made by the Examiner that such a conclusion by **Estell et al.** is "not persuasive or particularly pertinent." It is established that there must be a teaching, suggestion, or incentive supporting the combination. See, e.g., *In re Bond*, 910 F.2d 831, 15 USPQ2d 1566 (Fed. Cir. 1990) (PTO erred in rejecting the claimed invention as an obvious combination of the teachings of two prior art references when the prior art provided no teaching, suggestion or incentive supporting the combination.) That one of the combined references actually *teaches away* from the other reference is highly relevant to the issue of whether the references are properly combined. To ignore the reference that teaches away from the claimed invention demonstrates a clear reliance, on the part of the Examiner, on improper hindsight analysis of Applicants' claimed invention. Applicants respectfully assert that **Fahnestock et al.** and **Estell et al.** are not properly combined, particularly as applied to Claim 5.

The further combination of **Fahnestock et al.** and **Estell et al.** with the secondary references **TeNijenhuis** and **Suggs et al.** does not cure the deficiencies of the primary references. **TeNijenhuis** discloses the presence of indigenous alkaline protease in *Bacillus novo* species PB92. That reference is understood to merely disclose cultivating a *Bacillus* strain PB92 in a nutrient medium containing "the usual carbon, nitrogen and trace element sources under aerobic circumstances", and then "isolating the proteolytic enzyme formed from the fermentation broth." (See, Col. 1,

lines 63-68) **Suggs et al.** teach general methods for performing mutagenesis. Neither reference provides the missing teachings of the primary references, as described above. Accordingly, Applicants assert that the pending claimed invention is patentably distinct over the cited references.

> Regarding the claims (17, 19 and 24-25) directed to the protease itself, methods of making detergent compositions and using laundry processes containing such, these would have been obvious extensions of the rejected claims above as well. The protease of claim 17 does not initially appear to appreciably and patentably differ from the natural protease described by TeNijenhuis, wherein natural mutations of "at least one amino acid" would be expected to occur. Further, the simple production of the protease from the method/strain claims rendered obvious above would not have involved an inventive step, and would have been obvious from the methods themselves. Also, TeNijenhuis describe washing and detergent compositions and methods of cleaning/laundry processes using the high alkaline protease of strain PB92. Thus, the incorporation of the claimed protease into such compositions and methods would have been obvious given the teachings of TeNijenhuis.

The claimed mutant high alkaline protease recited in claim 17, the detergent of claim 19, and the methods of 24-25 each incorporate the limitations of the method of Claim 23. That claimed method, as discussed above, is patentably distinct over the cited references. Accordingly, the products manufactured using the novel method also are patentably distinct from products made using methods disclosed in the cited references.

The PB92 strain disclosed in the **TeNijenhuis** reference is not the same as Applicants' mutant of *Bacillus* novo species PB92, since the PB92 disclosed in that reference was not made using Applicants' claimed method of Claim 23. Specifically, there is no suggestion of obtaining an alkalophilic *Bacillus* strain "having no detectable extracellular high alkaline protease", or of transforming an alkalophilic *Bacillus* strain with "a cloning vector

comprising the 5' and the 3' flanking regions but not the coding region" of the gene coding for the high alkaline protease. Nor is there any suggestion of an alkalophilic *Bacillus* strain producing a mutant high alkaline protease which is "substantially free of expression product of an indigenous extracellular alkaline protease gene." Nor is there any suggestion of a method for production of a mutated high alkaline protease including the step of growing "an alkalophilic *Bacillus* strain host substantially incapable of reversion and having no detectable indigenous extracellular protease as a result of *deletion* of the gene." [emphasis added]

Accordingly, there is nothing in the combination of cited references to teach or suggest Applicants' claimed invention, as set forth in the pending claims, as amended. Applicants' therefore assert that the claimed invention is patentably distinct over the cited references.

CONCLUSION

In view of the foregoing amendments and remarks, Applicants submit that all of the pending claims now are in condition for allowance, which action is respectfully requested.

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If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney at (415) 926-6205.

Respectfully submitted,

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Barbara Rae-Venter, Ph.D.
Reg. No. 32,750

Weil, Gotshal & Manges
2882 Sand Hill Road, Suite 280
Menlo Park, CA 94025

Telephone: 415/926-6205
Facsimile: 415/854-3713

BRV:EFE:jgd